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Origins, evolution, domestication and diversity of *Saccharomyces* beer yeasts

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Yeasts have been used for food and beverage fermentations for thousands of years. Today, numerous different strains are available for each specific fermentation process. However, the nature and extent of the phenotypic and genetic diversity and specific adaptations to industrial niches have only begun to be elucidated recently. In *Saccharomyces*, domestication is most pronounced in beer strains, likely because they continuously live in their industrial niche, allowing only limited genetic admixture with wild stocks and minimal contact with natural environments. As a result, beer yeast genomes show complex patterns of domestication and divergence, making both ale (*S. cerevisiae*) and lager (*S. pastorianus*) producing strains ideal models to study domestication and, more generally, genetic mechanisms underlying swift adaptation to new niches.

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Introduction

'Domestication' is a term that refers to artificial selection and breeding of wild species to obtain cultivated variants with enhanced desirable features that thrive in man-made environments, often at the cost of suboptimal fitness in natural settings. Several genotypic and phenotypic signatures of domestication have been described in crops, livestock and pets. These include genome decay, polyploidy, chromosomal rearrangements, gene amplifications and deletions, horizontal gene transfer and loss of genetic diversity due to bottlenecking [1,2]. Interestingly, similar phenomena are also observed in various microbial species, both prokaryotic and eukaryotic, that are linked to human food production.

Perhaps the most well studied model is the common brewer's and baker's yeast, *Saccharomyces cerevisiae*, which is the main driver in many industrial fermentations. However, studies focusing on the evolution of industrial *Saccharomyces* strains often use the terms 'adaptive evolution' or 'domestication' too freely. For example, both terms are commonly used to explain phenotypic divergence from wild ancestors, overlooking alternative explanations such as random genetic drift [3]. Only recently, more elaborate studies have reported clear genome-wide signatures of domestication as well as convergent evolution of industrially relevant traits in separate lineages. These observations provide conclusive evidence that industrial yeast diversity is not solely shaped by genetic drift caused by bottlenecking and small isolated populations, but also as a result of selection and niche adaptation. In wine yeasts for example, adaptive horizontal gene transfer events [4,5,6•] and copy number variations [7,8,9,10] have been described that increase sugar and nitrogen metabolic activity, conferring competitive advantages during grape must fermentation and providing better tolerance to chemicals used in vineyards (e.g. copper sulphate) and in wine [11] (e.g. sulphite) (For a review see [7]). Interestingly however, the strongest genetic and phenotypic signatures of domestication are found in yeasts used for beer production. Several distinctive features make traditional beer production an ideal setting for microbial domestication. Firstly, beer yeasts are harvested and re-used after the fermentation process to initiate the next fermentation batch, a process called 'backslopping'. This continuous growth in a very specific industrial niche has resulted in continuous selection imposed by the brewing environment. Secondly, beer is produced year-round, causing a near-complete isolation from wild isolates. In contrast, wine is seasonal and wine yeasts spend most of the year in and around the vineyards or in the guts of insects, where nutrient limitation can trigger sexual cycles and hybridization with wild yeasts [12]. Therefore, present-day beer yeasts can be considered the result of a centuries-long evolution experiment in a highly selective niche. In this review, we will

highlight new insights into beer yeast evolution and domestication. We will discuss *S. cerevisiae* and *S. pastorianus*, both involved in production of specific beer types, which underwent a different route to domestication.

Domestication of *Saccharomyces cerevisiae* ale beer yeasts

Saccharomyces cerevisiae is the main microbial workhorse for the production of ale beers, which includes beer styles such as stouts, pale ales, doubles, triples and quadruples. As with all domesticated organisms, in *S. cerevisiae* the phenotypes of domesticated strains are a combination of enhanced selectable traits inherently present in *S. cerevisiae* (e.g. adaptation to sugar-rich, oxygen-limited environments and high tolerance to ethanol), and traits acquired during interaction with humans (e.g. efficient maltotriose utilization). In this review, we will expound on the latter aspect, and we refer to other review papers for the former [13,14].

Phylogenetics and population structure

Many studies of *S. cerevisiae* population structure focused on wine, wild and/or clinical strains, neglecting the broad diversity of beer yeasts. However, two recent studies, sequencing more than 100 ale beer strains, have provided the first comprehensive insight into their evolution and diversification [15••,16••]. Both studies found that the majority of beer yeasts are genetically distinct from known wild stocks, and cluster into two independent lineages (Figure 1). It has been estimated that the last common ancestor (LCA) of each lineage occurred around 1600–1700AD, well after the first reported beer production (3000–4000 BC), but before the discovery of microbes in the 19th century. Interestingly, the estimated period of occurrence of these LCAs coincides with the gradual switch from domestic brewing in private households, to more professional large-scale brewing, first in pubs and monasteries, and later in breweries. This suggests that true domestication of yeast occurred far more recently than the first leveraging of yeast for the production of fermented food and beverages, which likely happened several thousand years ago [17].

Genome structure

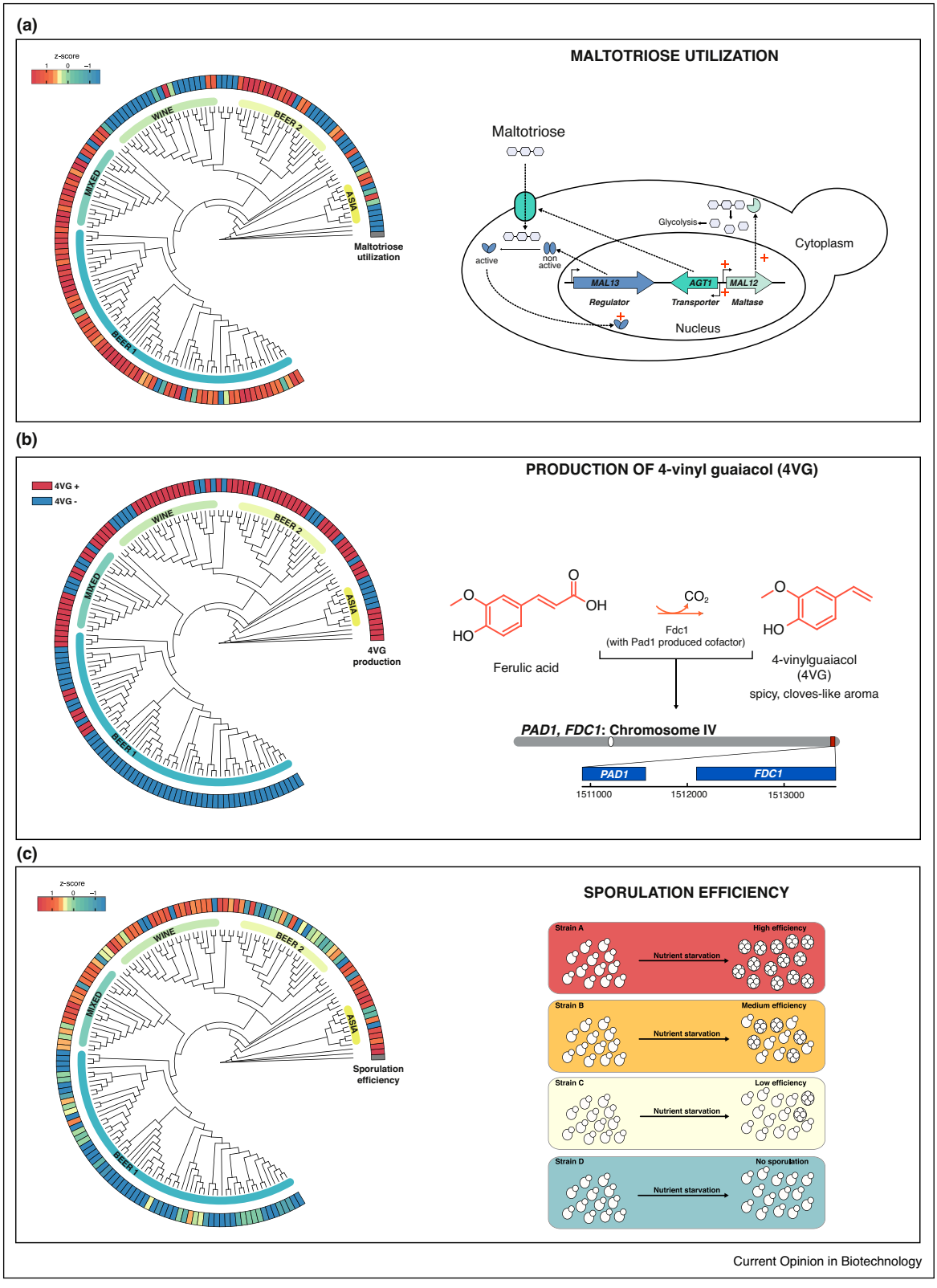
Variation in genome structure, including changes in ploidy and large segmental duplications or copy number variations (CNVs), have repeatedly been found in association with adaptation to specific niches in experimentally evolved microbes [18••,19]. Perhaps not surprisingly, similar chromosomal changes are also a recurrent theme in domestication of higher organisms, especially in crop species, for which polyploidization promoted the proper genetic circumstances to domestication [20]. Similarly, *S. cerevisiae* beer yeasts show large-scale genome structure variations. While most wild *S. cerevisiae* strains are clean diploids with very few large segmental duplications, the ploidy of the vast majority of beer strains exceeds 2n, with most close to 4n (Figure 2a). However, most ale yeasts also show aneuploidies, and are almost never perfectly diploid or tetraploid (Figure 2b). Previous studies have shown that aneuploidies and polyploidies can provide an adaptive advantage under selection [18••], but that they are often transient and are maintained until a more cost-effective adaptive strategy has evolved [21]. It has also been shown that small structural genome variations (e.g. duplication, deletion, recombination, gene conversion and rearrangement) are frequently located in telomeric and subtelomeric regions, which are known hotspots for evolution. These regions are functionally enriched for genes involved in nitrogen and carbon metabolism, ion transport and flocculation, and likely play a role in niche adaptation [15••,22,23•].

Brewing phenotypes

The most obvious sign of adaptation to a specific industrial niche is arguably the accentuation of traits desirable for humans that are a burden for the organism in a natural setting. Closer examination of the genetic underpinnings of specific traits have provided strong evidence that human selection indeed underlies certain industrially relevant traits in beer yeasts.

A prime example of a domestication trait in beer yeast is their ability to ferment maltotriose, an important carbon source in beer wort, but not generally found in high concentrations in natural yeast environments. Efficient metabolism of maltotriose imposes a selective advantage in brewing environments where it is present at high concentrations because it opens the door to a previously under- or poorly utilized energy source. This trait has evolved independently and through different genetic pathways in the two main beer lineages, suggesting strong selection pressure [15••] (Figure 1a). In one of the beer groups a homolog of the maltose transporter (called *AGT1*) with an increased affinity for maltotriose is present [24]. Interestingly, in the second beer group, the *AGT1* allele is non-functional, but the majority of isolates are able to efficiently ferment maltotriose, suggesting the presence of a distinct, yet unknown mechanism for the maltotriose uptake in this lineage. Another well-documented domestication trait is the selection against production of 4-vinyl guaiacol (4VG), an unpleasant aroma-active compound that is derived from ferulic acid, a cell wall component of barley. Yeast requires two genes for decarboxylation of ferulic acid to 4VG: *PAD1* and *FDC1*. Various independent nonsense mutations in these genes have been found in many industrial (and especially beer) yeasts, but not in biofuel or non-industrial isolates, suggesting that the selection of 4VG-free fermentations has favoured the spread of domesticated beer yeasts unable to produce this specific off-flavour (Figure 1b) [15••,16••].

Figure 1



Genetic and phenotypic diversity of industrial *S. cerevisiae* strains. In each panel, a cladogram depicting genetic relationship and a heatmap (surrounding the cladogram) depicting phenotypic behaviour of each strain is given on the left (data obtained from [15••]). On the right, a schematic depiction of the phenotypic traits is given. **(a)** Maltotriose utilization: beer yeasts show a significantly higher capacity to metabolize

Apart from human-driven selection for specific traits, domestication is often also accompanied by relaxation of selective constraints on traits that are not advantageous or too costly in man-made environments. This relaxed selection can result in gene loss or pseudogenisation of genes that are no longer required for survival, a process dubbed 'genome decay' [1]. In ale yeasts this is reflected by their inability to survive environmental and nutrient stress conditions not encountered during continuous growth in the nutrient-rich wort medium [15••]. One likely example of this phenomenon is represented by the lack of a functional sexual cycle in most ale beer isolates [15••] (Figure 1c), a trait with a crucial role in speeding adaptation under new, harsh conditions, but with limited added value in benign environments [25].

Domestication of *Saccharomyces pastorianus* lager beer yeasts

With over 90% market share, lager beer is by far the most popular beer style globally. The production process differs in many ways form ale beer brewing, but arguably the most profound distinction is that a different yeast species is used. Lager is brewed using *S. pastorianus*, which originates from the interspecific hybridization between *S. cerevisiae* and *S. eubayanus*, a closely related species that is not typically associated with industrial fermentations [26,27••,28•].

Phylogenetics and population structure

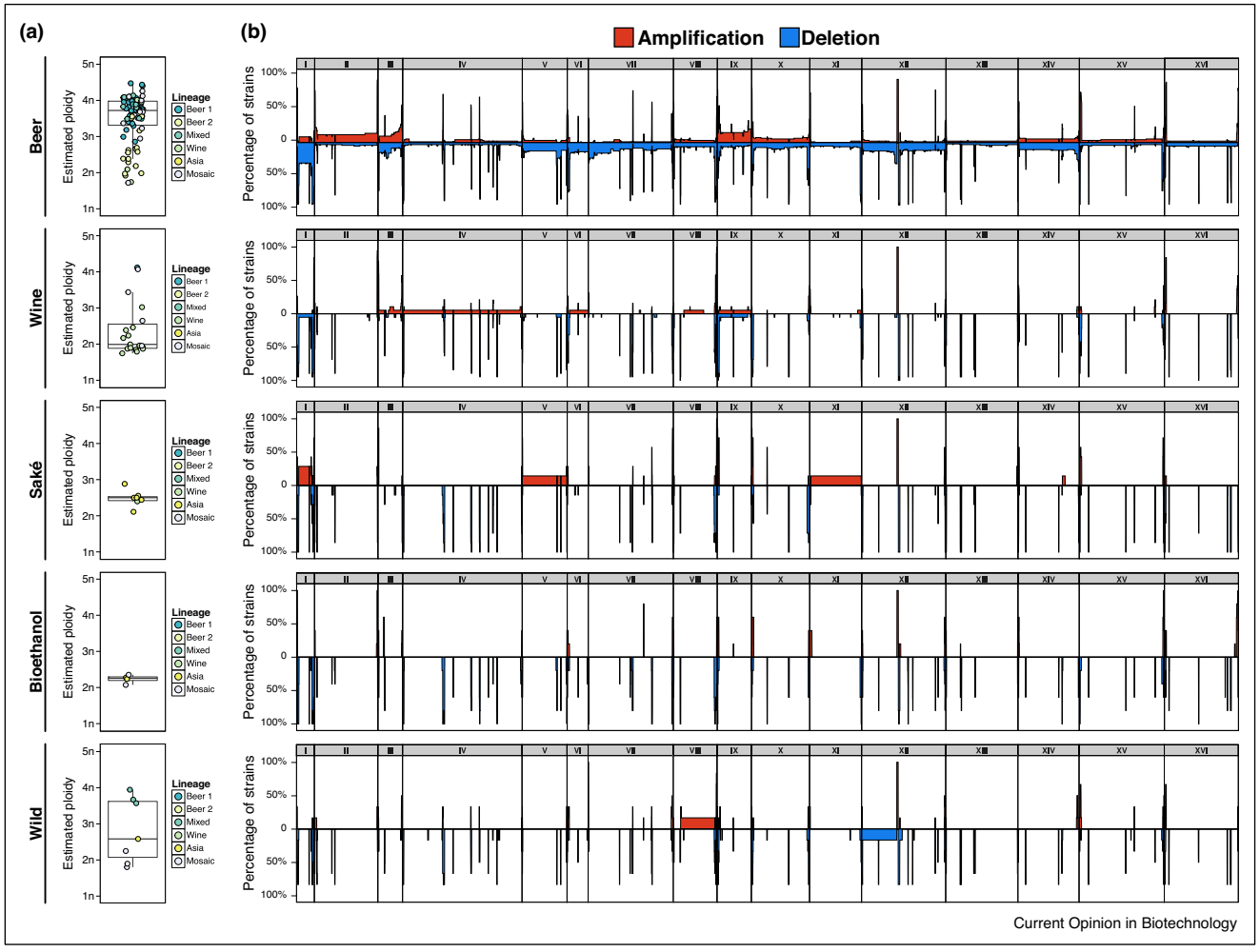
Lager strains are divided in two main distinct lineages, most commonly referred to as 'Saaz' (Type 1) and 'Frohberg' (Type 2). Whereas the existence of these two lineages suggests some degree of convergent domestication, the precise ancestry and evolution of the two *S. pastorianus* lineages is still controversial (for a review see [29]). Three main hypotheses for the *S. pastorianus* origin have been proposed, and different analyses have provided support for each. The most widespread hypothesis involves two completely independent hybridization events, each involving a different domesticated ale-type *S. cerevisiae* and a different wild *S. eubayanus* strain (Figure 3a). This hypothesis is supported by phylogenetic analysis, where the relative branch lengths for the S. *cerevisiae* and the *S. eubayanus* subgenomes are significantly different between the groups [27••], as well as by the pattern of loss or retention of subtelomeric regions in the *cerevisiae* part of the lager yeast genome, which are very different in Saaz and Frohberg yeasts [30]. However, other studies have identified several *S. eubayanus*/*S. cerevisiae* translocations that share identical breakpoints within the subgenomes of both Saaz and Frohberg lineages [31,32] (Figure 3b). Although it has been argued that identical breakpoints could have resulted from i) independent events at recombination hotspots or fragile sites in *Saccharomyces* chromosomes, or ii) events that occurred in one of the parental strains prior to the hybridization [27••], they might also indicate a shared hybridization event prior the divergence in the distinct lager lineages. A further hypothesis suggests a combination of the two scenarios; a single hybridization event between a haploid *S. cerevisiae* and a diploid *S. eubayanus* that led to an ancestral Saaz-like hybrid, followed by a second hybridization with a distinct haploid *S. cerevisiae* isolate that led to a Frohberg-like ancestral hybrid [33•] (Figure 3c).

Genome structure — interaction between subgenomes

Experiments where new interspecific hybrids are generated in the laboratory have shown that the genomes of newly formed hybrids tend to be highly unstable and undergo progressive genomic evolution until a more stable karyotype is reached. Hybrid genomic instability is probably due to an interplay between the relaxed selection on regions of genetic redundancy introduced by the hybridization event, selection acting on gene dosage balance, the potential co-adaptation of genes from the same parental genome and selection imposed by their growth environment [34,35,36,37]. Genomic changes include copy number variations [38] and partial or total chromosome loss [38], but also rearrangements between both subgenomes, resulting in chimaeric chromosomes [32]. Many of these mechanisms have been shown to allow adaptation in experimentally evolving interspecific hybrids. For example, loss-of-heterozygosity in newly developed interspecific hybrids is shown to be a reproducible adaptive strategy in low-nutrient environments, highlighting that hybrid genome resolution can be driven by positive selection acting on existing heterozygosity [39]. Chimaerism can be adaptive on the level of individual genes, for example, new interspecific *Saccharomyces* hybrids evolved in stringent nitrogen limitation recurrently evolve chimaeric *MEP1* alleles [40]. In lager yeasts, more than 20 chimaeric genes have been identified so far, including chimaeric variants of *ALD2* and *TDH2*, both involved in ethanol metabolism [32].

(Figure 1 Legend Continued) maltotriose, a prominent carbon source in beer medium. *AGT1*, a homolog of the sugar transporter *MAL11*, encodes for a permease with increased affinity to maltotriose compared to the wild type allele. *AGT1* is present in the Beer 1 and the Mixed clade, but absent and/or non-functional in the Wine and the Beer 2 clade. **(b)** Production of 4-vinyl guaiacol (4VG): 4VG is a spicy clove-like aroma compound, generally undesired in most fermented beverages. It is produced by yeast by the decarboxylation of ferulic acid, an abundant phenolic compound found in many plant cell walls, by the *FDC1*-encoded decarboxylase. This decarboxylase requires a flavin-derived cofactor produced by *PAD1*. *FDC1* and *PAD1* are clustered in the subtelomeric region of the right arm of chromosome IV. Many industrial yeasts, most notably the yeast in the Beer 1 clade, acquired loss-of-function mutations in *PAD1* and/or *FDC1*, resulting in a loss of the ability to produce 4VG [15••,16••]. **(c)** Sporulation efficiency: yeasts from the main beer lineages are generally obligate asexual or show low sporulation efficiency compared to strains from other clades.

Figure 2

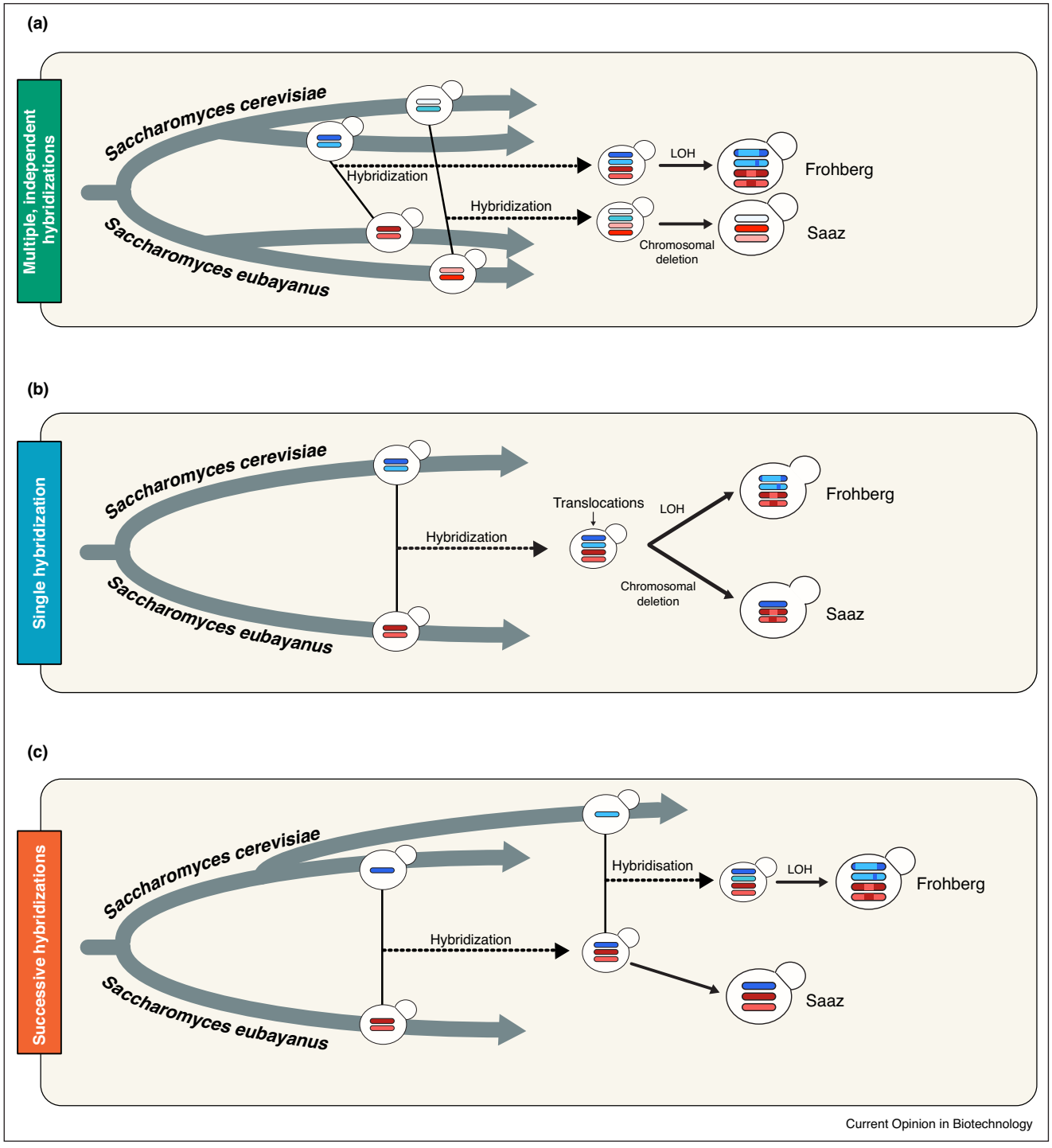


Ploidy variation of industrial yeast genomes. (a) Box plots representing the estimated ploidy of *S. cerevisiae* strains grouped by niche of isolation (beer, wine, saké, bioethanol, wild); colours indicate phylogenetic relationship (lineage) (cf. Figure 1-circular bands of the phylogenetic tree); strains with admixed ancestries, not belonging to any specific clean lineage, are indicated as 'mosaic'. Black lines represent median value and box edges are the 25th and 75th percentiles. Data obtained from [15••]. **(b)** Genome-wide visualization of CNV profiles compared to the reference strain S228C, aggregated across all strains originating from different niches; colours indicate amplification (red) and deletion (blue). Data obtained from [15••].

Brewing phenotypes

The dominance of *S. pastorianus* in the lager brewing industry suggests a strong selective advantage of the interspecific hybrid over their respective parental species. It has been argued that some parts of the *S. eubayanus* genome confer enhanced cold-tolerance, while the *S. cerevisiae* subgenome holds the advantage of other brewing adaptations, such as efficient fermentation, including the use of maltotriose. However, maltotriose transporters from *S. eubayanus* and not *S. cerevisiae* enable maltotriose utilization in some Saaz-type lager yeasts [41]. Conversely, certain *S. cerevisiae* strains show adaptation to cold environments [42], which implies that the cold-tolerance does not necessarily originate from *S. eubayanus*, and that the origin of lager yeasts may have a different foundation. Recent papers suggest that increased fitness of interspecific yeast hybrids can also be due to genetic incompatibilities that perturb safeguard mechanisms that would normally limit growth in the parental strains, leading to hybrids that divide more (and thus have a higher fitness) in stressful environments, such as beer wort at low temperatures [43]. However, more research is required to untangle the specific roles of lager yeast subgenomes in the brewing environment.

Figure 3



Current models for the origin of Frohberg and Saaz lineages. (a) Frohberg and Saaz groups originated from at least two independent hybridization events between distinct diploid *S. cerevisiae* and diploid *S. eubayanus* parental strains. **(b)** Frohberg and Saaz groups originated from a single hybridization event between a diploid *S. cerevisiae* and a diploid *S. eubayanus*. Translocations occurred in the ancestral hybrid prior to the divergence of the Saaz and Frohberg lineages and are shared between the two groups. After hybridization, the Frohberg lineage experienced loss of variation between intra-homologous chromosomes in the *S. cerevisiae* subgenome via loss of heterozygosity [33•] and the Saaz lineage lost roughly half of the *S. cerevisiae* derived chromosomes. **(c)** Frohberg and Saaz groups originated from at least one shared hybridization event between a haploid *S. cerevisiae* and a diploid *S. eubayanus*. The triploid ancestral hybrid further diverged into the Saaz lineage, and the Frohberg lineage arose by another hybridization event with a distinct haploid *S. cerevisiae* [33•].

Conclusions and outlook

Recent studies have demonstrated that beer yeasts have been domesticated by enduring growth in man-made fermentation environments. The strong selective pressure imposed over many generations has contributed to the emergence of desirable phenotypes, but has also dramatically affected the genomic structure and genome stability of the domesticates.

Interestingly, continuous 'backslopping' is not common practice anymore in most of today's commercial breweries. Instead, brewers dispose of their yeast culture after a few consecutive fermentations to start a new brew with a frozen stock culture. This continual reversion to the same yeast stock ensures consistency of their product, but prevents further evolution of the beer yeast. Therefore evolution and domestication of beer yeasts within breweries may have practically halted. However, the process has now moved to specialized labs, where the expanding experimental toolbox and the wealth of 'omics' data available for *Saccharomyces* yeasts opens new avenues to generate novel and superior industrial variants. Specifically, experimental evolution, similar to the process in traditional brewing, can be used in conjunction with techniques like crossing, marker-assisted breeding and mutagenesis to effectively generate and test new phenotypic variants and combinations [44,45]. Thus, several centuries after the dawn of beer yeast domestication by commercial-scale brewing, a second revolution, sparked by biotechnology, is now driving a new era of beer yeast evolution and domestication.

Conflicts of interest

None.

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